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Abstract

This study was undertaken to determine what effect if any that fluorescein, thimerosal, or EDTA would have on tear breakup time. It was found that fluorescein would significantly reduce tear breakup time in man. EDTA and thimerosal as administered in this study had no significant effect on tear breakup time in man.

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THE EFFECT OF FLUORESCEIN, EDTA, AND THIMEROSAL ON THE RATE OF TEAR BREAKUP IN MAN

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ABSTRACT

This study was undertaken to determine what effect if any that fluorescein, thimerosal, or EDTA would have on tear breakup time. It was found that fluorescein would significantly reduce tear breakup time in man. EDTA and thimerosal as administered in this study had no significant effect on tear breakup time in man.

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THE EFFECT OF FLUORESCEIN, EDTA AND THIMEROSAL ON THE RATE OF TEAR BREAKUP IN MAN

INTRODUCTION

Tear breakup time is defined as the time it takes for a dry spot to form on the cornea in the absence of a blink. The purpose of this study was to show the effect of three different chemicals on tear breakup time. These three were: sodium fluorescein, EDTA, and thimerosal. Benzalkonium chloride, a chemical widely used as an antimicrobial in ophthalmic solutions and contact lens solutions, has been shown to shorten the tear breakup time in animal and human subjects (Wilson, Duncan, & Jay, 1975).

The precorneal tear film is composed of three separate and distinct layers. They are: the inner mucin layer, the central aqueous layer, and the outer lipid layer. The mucin layer, which is secreted by the goblet cells of the conjunctiva, readily adheres to the cornea greatly increasing its wettability. When the cornea is cleaned of mucin it becomes hydrophobic (Lemp & Holly, 1970). The mucin layer is essential for the maintenance of the precorneal tear film. It provides a desireable interface between the cornea and the aqueous portion of the tears. The aqueous layer of the tear film, which is secreted by the accessory lacrimal glands, forms the major vehicle for wetting and nourishing the cornea. When there is an aqueous deficiency or a mucin deficiency of the tears, tear breakup time is reduced (Lemp, Dohlman, & Kuwabara, 1971). The lipid layer of the tears forms a thin barrier against evaporation of the underlying layers.

With the elimination of the lipid layer and the resultant increase in evaporation of the tears, tear breakup time is reduced. In a study done on rabbits, the elimination of the outer lipid layer resulted in a 15 fold increase in the rate of evaporation of the tears (Mishima & Maurice, 1961).

A cited method of measuring tear breakup time employs fluorescein as an aid in observing tear breakup (Lemp & Hammill, 1973). After the instillation of fluorescein in the eye by touching the bulbar conjunctiva with a moistened fluorescein strip, a bimicroscope with a cobalt blue light source is used to observe breakup of the tears. Any break is easily and readily seen as a distinct dark spot in contrast to the fluorescent tear layer that covers the rest of the cornea. A true break in the tears is distinguished from a thinning of the tear layer by the sharp distinctive borders that surround the break. Using the above technique the majority of breakup times in one study fell in the 15-34 second range (Lemp & Hammill, 1973).

Fluorescein is a yellowish red dye when in aqueous solution. It has the chemical formula of $C_{20}H_{12}O_5$. The sodium salt of fluorescein is used in aqueous solution in the eye as an aid in assessing the fit of contact lenses. Corneal abrassions can be better seen with the use of fluorescein because the fluorescein is absorbed by the damaged corneal cells. As previously mentioned, flourescein is also used in a method of measuring tear breakup time.

(Etheylenedinitrilo)-tetraacetic acid (EDTA) is a chelating agent, and is used as an antimicrobial in contact lens solution.

Merthiolate® (Thimerosal, N. F. Lilly) is a complex organomercurial salt consisting of approximately 49% mercury. Its chemical name is sodium ethylmercurithiosalicylate. Thimerosal is water soluble, and it has been used extensively as a presevative in biological preperations and pharmaceutical products. It is also used as a preservative agent in contact lens solutions.

SUBJECTS

All of the subjects but one were college students in the 20 year to 30 year age group. The other subject was the 24 year old wife of a college student. Two of the subjects were female.

METHODS AND MATERIALS

Three experimental chemical solutions and one control solution were used in this experiment. The control solution contained NaCl in 0.9% concentration, and sodium fluorescein in 0.2% concentration. The thimerosal experimental solution contained thimerosal in 0.004% concentration, NaCl in 0.9% concentration, and sodium fluorescein in 0.2% concentration. The experimental sodium fluorescein solution contained sodium fluorescein in 2.0% concentration, and NaCl in 0.9% concentration. The experimental EDTA solution contained EDTA in 0.1% concentration, NaCl in 0.9% concentration, and sodium fluorescein in 0.2% concentration. The thimerosal and EDTA solutions differed from the control solution only in their content of the experimental chemicals. The 2.0% sodium fluorescein solution differed from the control solution only in the concentation of sodium fluorescein contained, there being a ten fold difference in the concentration.

Sodium fluorescein was used in the control solution, the thimerosal solution, and the EDTA solution as an aid in observing tear break-up. All of the above solutions were aqueous solutions.

Some subjects served as subjects for all three experimental solutions. Others served as subjects for only two of the experimental solutions, and others served as subjects for only one experimental solution.

The experimental procedure was the same for each of the experimental solutions tested. All of the solutions were steam heat treated each day prior to testing to destroy all vegetative forms of yeast, bacteria, and molds.

The solutions were administered by instilling two drops in the eye. The excess solution was blotted away with tissue. The subject blinked several times, then held the eyes open while the eye under study was observed with a biomicroscope. The time for the tears to breakup was then measured. In the testing sequence the right eye was always tested first, receiving either the control solution or the experimental solution. Then the left eye received the other solution, e.g. if the right eye received the control solution, then the left eye received the experimental solution. After a five to ten minute wait the procedure was repeated with the right eye receiving the other solution and then the left eye receiving the other solution. If no break in the tears was observed after 60 seconds the measurement was terminated.

RESULTS

Student's paired "t" test for a repeated measures design was used to anylyse the data. There was a significant difference in the effect of the control solution and the effect of the experimental fluorescein solution on tear breakup time. The difference was significant at the 0.001 level of confidence. In comparing the effect in only those eyes which received the control solution first, and the experimental fluorescein solution second, the difference was significant at the 0.02 level of confidence. In comparing the effect in only those eyes which received the experimental fluorescein solution first and the control solution second, the difference was significant at the 0.05 level of confidence. In all of the above cases the tear breakup time was lower for the experimental fluorescein solution than it was for the control solution (see table no. 1).

CONDITIONS OF COMPARISON	N	TEAR BREAKUP TIME IN SECONDS (MEAN ± STANDARD DEVIATION)		LEVEL OF SIGNIFICANCE
		CONTROL	FLUORESCEIN	
ALL OF EXPERIMENTAL VS. ALL OF CONTROL	30	21.2±16.9	8.9±7.0	0.001
EYES RECEIVING EXPERIMENTAL FIRST	15	24.7±16.9	9.0±7.2	0.05
EYES RECEIVING CONTROL FIRST	15	17.8±14.1	8.8±7.0	0.02

Table no. 1 Effect of Fluorescein on Tear Breakup Time.

There was no significant difference between the effect of the control solution versus the effect of the EDTA solution. In comparing the effect on only those eyes which received the EDTA solution first and the control solution second, there was no significant difference in the two treatments. In comparing the effect on only those eyes which received the control solution first and the EDTA solution second, there was no significant difference in the two treatments (see table no. 2).

CONDITIONS OF COMPARISON	N	TEAR BREAKUP TIME IN SECONDS (MEAN ± STANDARD DEVIATION) CONTROL	EDTA	LEVEL OF SIGNIFICANCE
ALL OF EXPERIMENTAL VS. ALL OF CONTROL	22	29.0±22.9	29.6±21.1	NOT SIGNIFICANT
EYES RECEIVING EXPERIMENTAL FIRST	11	34.5±21.0	32.4±19.6	NOT SIGNIFICANT
EYES RECEIVING CONTROL FIRST	11	23.1±24.4	26.7±23.1	NOT SIGNIFICANT

Table no. 2 Effect of EDTA on Tear Breakup Time

There was no significant difference between the effect of the control solution versus the effect of the thimerosal solution. In comparing the effect on only those eyes which received the thimerosal solution first and the control solution second, there was no significant difference in the two treatments. In comparing the effect on only those eyes which received the control solution first and the thimerosal solution second, there was no significant differenc in the two treatments (see table no. 3).

CONDITIONS OF COMPARISON	N	TEAR BREAKUP TIME IN SECONDS (MEAN ± STANDARD DEVIATION) CONTROL	THIMEROSAL	LEVEL OF SIGNIFICANCE
ALL OF EXPERIMENTAL VS. ALL OF CONTROL	20	21.5±15.7	23.0±19.3	NOT SIGNIFICANT
EYES RECEIVING EXPERIMENTAL FIRST	10	23.5±16.7	21.9±17.5	NOT SIGNIFICANT
EYES RECEIVING CONTROL FIRST	10	19.5±15.3	24.1±21.7	NOT SIGNIFICANT

Table no. 3 Effect of Thimerosal on Tear Breakup Time.

In comparing the effect of all of the first treatments versus all of the second treatments for each of the experimental conditions, there was no significant difference between the two. Or, for each of the experimental conditions, the first treatment did not significantly shorten nor lengthen the breakup times of the second treatment.

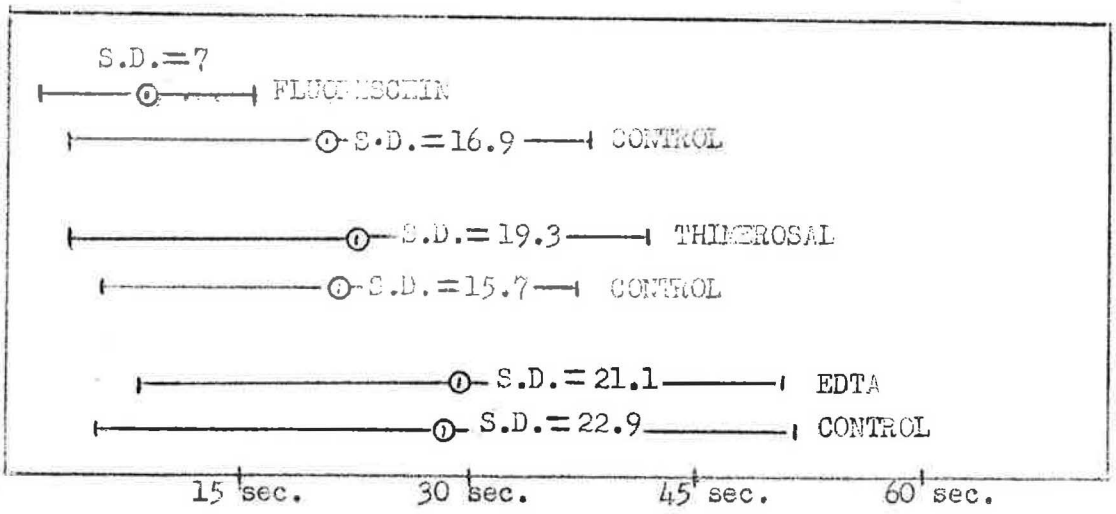

Figure no. 1 Graph comparing mean breakup times of the three experimental solutions and their respective control solutions.

DISCUSSION

Sodium fluorescein shows a remarkable ability to reduce tear breakup time in human subjects. This would indicate that the use of fluorescein in measuring tear breakup time warrants further consideration. The use of even 0.2% sodium fluorescein in this study yeilded a mean breakup time four times lower than the mean breakup time as measured in a study where saline alone was instilled prior to the tear breakup measurements (Wilson, Duncan, & Jay, 1975). Perhaps this difference could be attributed to the difference in the ability to detect the first break in the precorneal tear film in the two different methods. This would assume that tear breakup would have been detected earlier with the use of fluorescein. However, the use of fluorescein in measuring tear breakup time does need to be standardized. The differences in various brands of fluorescein strips, and the amount of fluorescein instilled from the strip could have an effect on tear breakup time.

The use of thimerosal and EDTA in contact lens solutions, when considered in the light of this study, would have no detrimental effect on the integrity of the precorneal tear film.

REFERENCES

Lemp, M. A., Dohlman, C. H., and Kuwabara, T. (1971) Dry Eye Secondary to Mucus Deficiency. American Academy of Ophthalmology and Otolaryngology. Vol. 75 No. 6, pp. 1223-1227.

Lemp, M. A., and Holly, F. J. (1970) The Precorneal Tear Film. Archives of Ophthalmology. Vol. 83 (Jan), pp. 89-94.

Lemp, M. A., and Hammill, R. J. (1973) Factors Affecting Tear Film Breakup in Normal Eyes. Archives of Ophthalmology. Vol. 89 (Feb), pp. 103-105.

Mishima, S., and Maurice, D. M. (1961) The Oily Layer of the Tear Film and Evaporation from the Corneal Surface. Experimental Eye Research. Vol. 1 No. 1, pp. 39-45.

Wilson, W. S., Duncan, A. J., and Jay, J. L. (1975) Effect of Benzalkonium Chloride on the Stability of the Precorneal Tear Film in Rabbit and Man. British Journal of Ophthalmology. Vol. 59 No. 11, pp. 667-669.